

Kinetic aspects of the interaction of blood clotting enzymes

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Kinetic Aspects of the Interaction of Blood Clotting Enzymes

V. The Reaction Mechanism of the Extrinsic Clotting System as Revealed by the Kinetics of One-Stage Estimations of Coagulation Enzymes

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Introduction

In the preceeding articles we discussed the relationship between clotting time and the concentration of clotting factors II, VII, and X when varied simultaneously. Clotting time was found to be rectilinearly dependent upon the inverse of clotting-factor concentration (16, 17).

We have indicated the striking similarity between this relationship and the rectilinear relationship frequently observed between the inverse of the reaction velocity and the inverse of substrate concentration in simple enzymatic reactions. The Briggs-Haldane theory of steady-state enzyme kinetics explains this relationship for these simple reactions (9). This theory, however, cannot be applied without modification to systems in which coagulation factors interact, because the assumption that the substrate is present in excess over the enzyme, which is one of the basic assumptions of this theory, is very probably not justified in coagulation systems. This does not disturb the general concept because deletion of this assumption does not disturb the rectilinear relationship between the inverse of reaction velocity and the inverse of substrate concentration (14). Therefore, the possibility must be left open that the relationship found between coagulation-factor concentration and clotting time is dependent upon a reaction of the Briggs-Haldane type occurring in the coagulation reaction sequence.

Recognition of this possibility compels an inquiry into the mechanism that causes the intricate reaction sequence of blood coagulation to follow such seemingly simple rules.

In the experiments reported in this article we first of all attempted to establish the relationship between the concentration of any one of the factors of the extrinsic coagulation system (factors II, V, VII, and X) and the clotting time in a one-stage test specific for that factor. In the discussion we shall elaborate upon the mechanism that can explain the relationships found.

Preliminary experiments indicated that a modification of the cascade scheme (23) is necessary to explain our findings.

It might be possible that the theory on blood coagulation proposed by Seegers (26, 27) would give a ready explanation of our findings. This possibility cannot be taken into account because the present author has not been able to construct a reaction

scheme of blood coagulation based on Seegers' theory and permitting kinetical treatment, this theory being always explained in diagrams of the flowchart type rather than in terms of chemical reactions. Although Dr. Seegers was kind enough to enlarge on his ideas in a personal letter, we did not obtain sufficient information to arrive at a definite series of reaction formulae such as would be necessary for our purposes.

An interpretation of our data will prove to be impossible without the consideration of the action of inhibitors or inactivators of the active procoagulants. The introduction of these substances in our reaction schemes seems justified, because the system in which we carry out our estimations is not essentially purer than whole plasma is, and because in plasma many natural inhibitors or inactivators have been recognized (1, 2, 7, 8, 10, 11, 13, 21, 22, 29, 30, 31).

Materials and Methods

The one-stage measurements were carried out as already described. The reagents have also been described in the preceeding articles (16, 17, 18).

A factor II reagent containing all the necessary procoagulants (i. e. factors I, V, VII, and X) but lacking Antithrombin III was prepared as follows:

The factor II reagent was diluted twentyfold with twice-distilled water, after which CO_2 was led through the solution for 15 min. The resulting precipitate was collected by centrifugation for 10 min at 12,500 g and dissolved in Michaelis buffer (pH 7.4) to half the volume of the original reagent. The concentration of clotting factors present in this solution was then estimated. The ratio of the concentration of the factors I, V, VII, and X did not differ significantly from that in the original material, but the absolute level was higher than that of the original reagent. By further dilution with Michaelis buffer, the solution was adjusted to the same level of coagulation factors as that of the original reagent. In this solution the concentration of Antithrombin III was less than 0.5% of that of the original material. Antithrombin III was estimated according to Hensen (21).

All experimental points given in the graphs are the means of at least 12 estimations unless otherwise indicated.

Experimental Results

All the experiments reported here were so-called one-stage coagulation factor determinations in the extrinsic system. The objective of these determinations was to assess the concentration of one factor (F), independent of the other factors; therefore, a reaction mixture in which the concentration of F would be rate-limiting was required. This reaction medium had the following composition:

0.1 ml *reagent*. This reagent is a plasma containing the smallest feasible amount of F, all other factors being present in relatively high concentration. For the sake of argument, we will assume these other factors to be present in concentrations equal to the concentration in normal plasma. The minute amount of F present in the reagent we will call L.

0.1 ml *tissue thromboplastin*; providing necessary phospholipid and tissue factor (human brain thromboplastin according to Owren & Aas).

0.1 ml *sample*, containing between 1 to 10% F as well other clotting factors.

The reaction is started by addition of 0.1 ml CaCl_2 25 mM.

In reaction mixtures of the above composition the final concentration of F will vary between $\frac{1}{4}(L + 1)\%$ and $\frac{1}{4}(L + 10)\%$. The concentrations of the other clotting factors vary between $\frac{1}{4}(100 + 1)\%$ and $\frac{1}{4}(100 + 10)\%$. The variation in these concentrations is brought about by the variations in the sample. Because $L \ll 100\%$, the concentration of F will be much lower than the concentration of the

other clotting factors. Since the absolute value of the variation is the same for all clotting factors (at least when dilutions of a normal plasma are tested, as is the case in the experiments described here), this means that the percentage variation in concentration is by far the greatest for factor F, and that the concentration of factor F is always appreciably smaller than that of the other factors. Consequently, the variation in clotting time will be essentially a function of the variation in the concentration of the added F and not of that of the other clotting factors. This, by the way, does not imply that major changes in the final concentration of one of the other clotting factors would not influence the clotting time.

That the amount of F present in the reagent (L) is not negligible can be seen when buffer is added to the reaction mixture described above instead of a sample containing some concentration of F. A distinct—although long—clotting time is then obtained (t_c).

For factor II estimations, we have already shown (14, 18) that, with the *a priori* assumption that steady-state kinetics can be applied to the reaction under study, the magnitude of L can be computed from the slope of a curve obtained by plotting t_c against $(t_1 - t_c):C$ (C = amount of factor II added with the sample).

The fact that this curve could be demonstrated to be a straight line, as was predicted by steady-state kinetics, in combination with the fact that the plot of clotting time against the inverse of the total concentration ($L + C$) of factor II also was a straight line, was considered as supportive evidence *a posteriori* of the assumptions made *a priori* (18).

We then tried to find out whether the same way of handling data would give useful results in experimental situations where clotting factors other than factor II were made rate-limiting. Figs. 1–6 give the results obtained from experiments in which each of the factors of the extrinsic system was made rate-limiting. For any of these factors, clotting time proved to be a linear function of the inverse of the clotting-

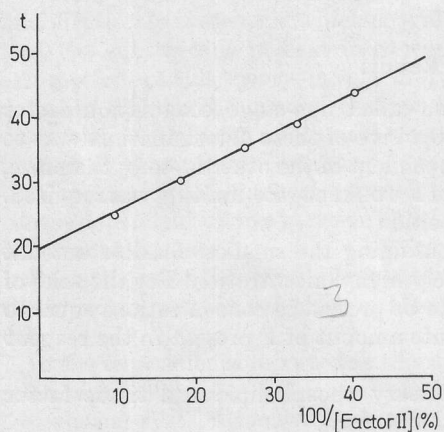


Fig. 1.

Fig. 1. The relation of factor II concentration to clotting time. Reaction medium: 0.1 ml congenital factor II-deficient plasma, 0.1 ml normal plasma (diluted 1 in 10 to 1 in 50), 0.1 ml thromboplastin, and 0.1 ml CaCl_2 33 mM. The points represent the means of 25 estimations.

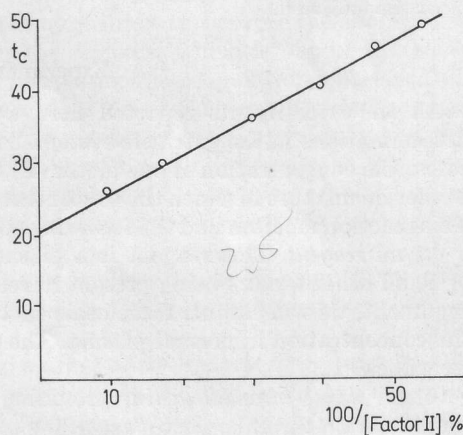


Fig. 2.

Fig. 2. The relation of factor II concentration to clotting time. Reaction medium: 0.1 ml artificial factor II-deficient plasma, 0.1 ml normal plasma (diluted 1 in 10 to 1 in 50), 0.1 ml thromboplastin, and 0.1 ml CaCl_2 33 mM. The points represent the means of 25 estimations.

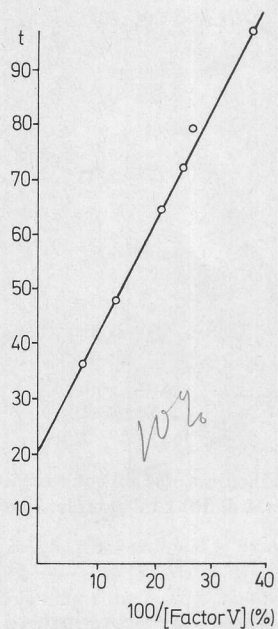


Fig. 3.

Fig. 3. The relation of factor V concentration to clotting time. Reaction medium: 0.1 ml Ba-stearate absorbed normal plasma, 0.1 ml thromboplastin, 0.1 ml CaCl_2 25 mM and 0.1 ml sample. The points represent the means of 25 estimations.

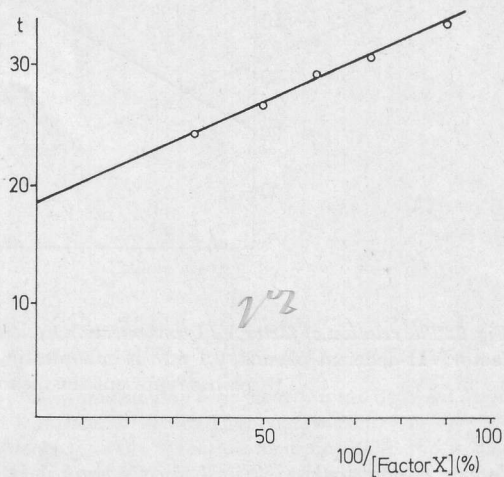


Fig. 4.

Fig. 4. The relation of factor X concentration to clotting time. Reaction medium: 0.1 ml congenital factor X-deficient plasma, 0.1 ml thromboplastin, 0.1 ml CaCl_2 25 mM and 0.1 ml sample. The points represent the means of 25 estimations.

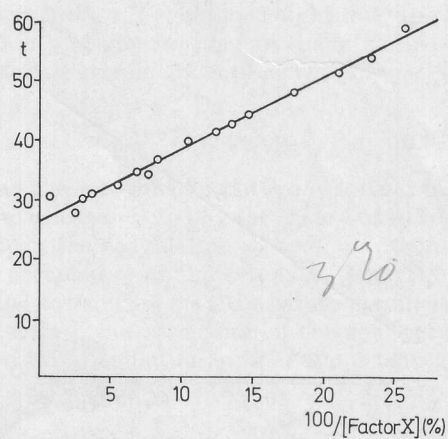


Fig. 5. The relation of factor X concentration to clotting time. Reaction medium: 0.1 ml factor X deficient plasma from a patient with amyloidosis, 0.1 ml thromboplastin, 0.1 ml CaCl_2 25 mM and 0.1 ml sample. The points represent the means of 10 estimations.

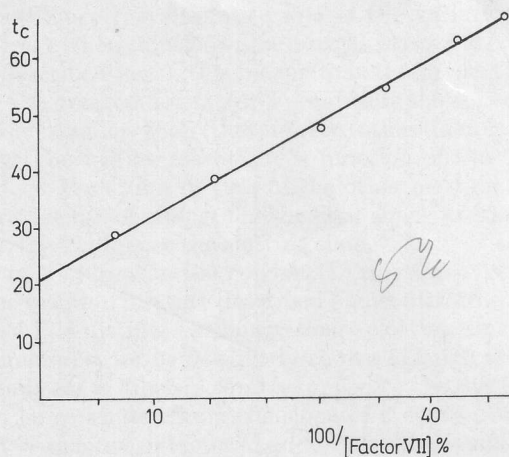


Fig. 6. The relation of factor VII concentration to clotting time. Reaction medium: 0.1 ml congenital factor VII-deficient plasma, 0.1 ml thromboplastin, 0.1 ml CaCl_2 25 mM and 0.1 ml sample. The points represent the means of 25 estimations.

factor concentration. Fig. 7 shows that it is not possible to apply our procedure to the calculation of L in an experiment employing a reagent deficient in both factor VII and factor X. The method is also not applicable to data from estimations in the intrinsic system (i. e. factors VIII, IX, and XII). Fig. 8 shows that the rectilinearity in a reciprocal plot of a factor II estimation is consistent over the whole range for which testing is feasible, whereas the rectilinearity in a conventional double logarithmic plot only holds within certain limits.

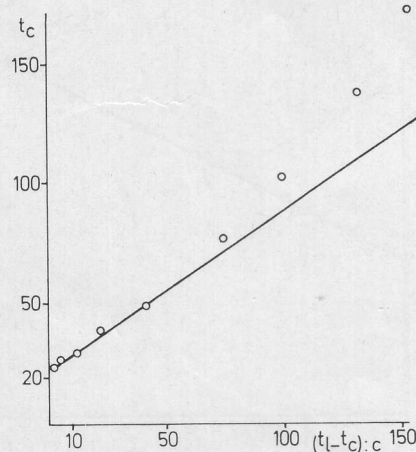


Fig. 7. The correction plot obtained with a reagent plasma deficient in both factors X and VII. The experimental data do not fit to a straight line in this case, the plot can therefore not be used to calculate L. Reaction medium: 0.1 ml Seitz filtered bovine oxalated plasma, 0.1 ml thromboplastin, 0.1 ml CaCl_2 25 mM and 0.1 ml sample. The points represent the means of 25 estimations.

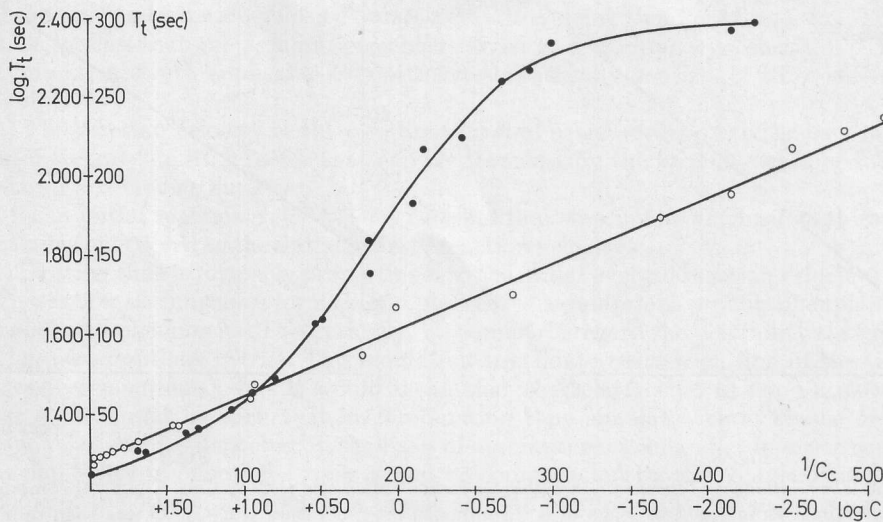


Fig. 8. The relationship between factor II concentration and clotting time plotted in two different ways. ●-----● logarithm of clotting time against logarithm of factor II concentration. ○-----○ clotting time against the inverse of factor II concentration. Reaction medium: as in Fig. 2. The points represent the means of 3 estimations. The inverse of C_c is given in arbitrary units, so that $1/C_c = 200$ when $C_c = 10$; T_t = clotting time.

Fig. 9 shows that a rectilinear relationship can also be demonstrated when clotting time is plotted against the inverse of the concentration of the prothrombinase¹) generated in a two-stage test according to Biggs (2).

As can be seen from Fig. 10, the absence of Antithrombin III does not disturb the rectilinear relationship found in factor II estimations. Fig. 11 shows that in a restricted concentration range, i. e. in a range where thromboplastin is not present in amounts high enough to have an inhibitory action, the plot of clotting time against the inverse of thromboplastin concentration is again a straight line. In this experiment, haemophilic plasma was used as a reagent to prevent the intrinsic coagulation system from interfering with the extrinsic system under observation.

Discussion

The observation that the relationship between clotting factor concentration and clotting time follows the rules predicted by the theory of steady-state enzyme kinetics if the differences between the coagulation system and a simple enzymatic reaction are overlooked, can be accepted as an indication that enzyme kinetics may be applied to the results of clotting tests. This may constitute a sufficient basis for the design of the experiments described, but discussion of the results requires a recognition of the differences between the coagulation system and a reaction that can be directly described in terms of steady-state enzyme kinetics.

1) The name prothrombinase is used to indicate the activity that directly acts upon prothrombin to form thrombin. The enzymatic nature of this action seems to be sufficiently proven (15, 25) to justify the suffix-ase in this context.

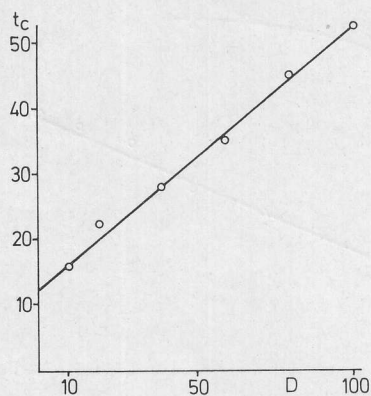


Fig. 9.

Fig. 9. The relationship between prothrombinase concentration and clotting time. The prothrombinase was obtained as in the first stage of a thromboplastin generation test (1). It was then diluted, after which the clotting time in the second stage was determined; clotting time was plotted against dilution (i. e. the inverse of the concentration). D = dilution.

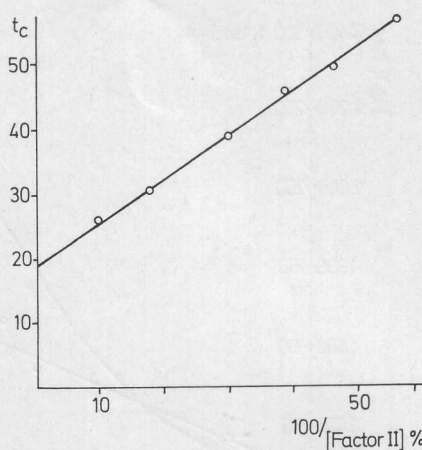


Fig. 10.

Fig. 10. The relation of factor II concentration to clotting time in a Antithrombin III-free medium. Reaction medium: as in Fig. 2 but with Antithrombin III-free reagent and normal plasma.

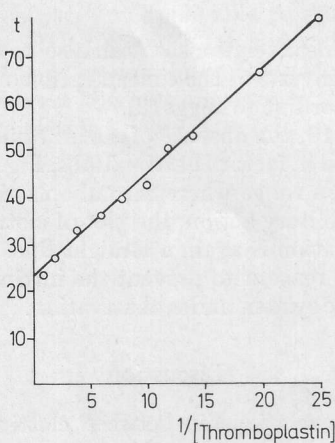


Fig. 11. The relationship of thromboplastin concentration to clotting time. Reaction medium: 0.2ml platelet-free factor VIII-deficient plasma, 0.1 ml thromboplastin dilution (diluted 1 in 1 to 1 in 25), and 0.1 ml CaCl_2 33 mM.

In the process linking a coagulation-factor concentration in a rate-limiting step to the final coagulation time in the complete system, three stages can be distinguished:

1. The concentration of the rate-limiting factor gives rise to a reaction velocity in the rate-limiting step.
2. The reaction velocity in the rate-limiting step causes a certain over-all reaction velocity, also determined by the concentrations of all procoagulants and anticoagulants present in the reaction mixture.

3. The over-all reaction velocity causes a certain clotting time.

This implies that the assumption which served as a working hypothesis, i. e. that enzyme kinetics are applicable to clotting tests, actually consists of three different assumptions:

a) The reaction velocity in the rate-limiting step bears a relation to the concentration of the rate-limiting factor that can be described by the formulae obtained from the theory of enzyme kinetics.

b) The initial reaction velocity of a rate-limiting step in the extrinsic pathway is linearly proportional to the initial over-all reaction velocity.

c) Clotting time is inversely proportional to the initial over-all reaction velocity.

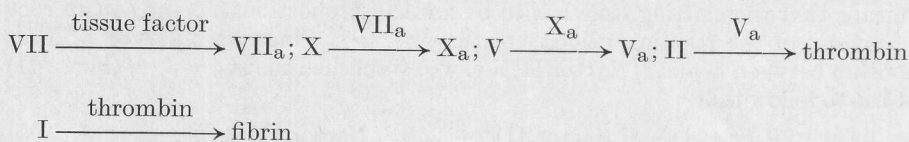
These three assumptions form the simplest set of assumptions capable of explaining the observed phenomena. Theoretically, we cannot disregard the possibility that none of these assumptions is true. This would not invalidate the recognition of the three stages just mentioned, but it would mean that the relationship in two or more of these steps would be such that in combination they simulate the existence of the simple relationships expected on the basis of assumptions a, b, and c. In other words, the complexity of one of the steps would be counteracted by the complexity of one or more of the others, resulting in a simple over-all picture. It will be shown that this must be considered improbable.

We may now consider the three assumptions separately. Assumption a) cannot be considered unlikely because there is no reason why enzymatic reactions in blood coagulation should not behave in the same way as all other enzymatic reactions do.

At the moment, we can give no direct proof of assumption c). In experiments in which the prothrombinase concentration in mixtures of factor X_a , factor V, phospholipid, and Ca ions is assessed directly by measuring the velocity of thrombin generation from an excess of prothrombin, the inverse of the prothrombinase¹⁾ concentration bears a linear relationship to the inverse of the concentration of any of the constituent factors (15). When factor X (Figs. 4, 5), factor V (Fig. 3) or phospholipid (Fig. 11) are made rate-limiting in a one stage test, prothrombinase can be accepted to be rate-limiting. As can be seen from the figures t_c bears a linear relationship to the inverse of the rate-limiting factor here. In combination with the data from ref. 15 this can be accepted as suggestive evidence for the assumption that clotting time is proportional to the inverse of prothrombinase concentration in experiments in which this concentration is rate-limiting; therefore it supports our assumption.

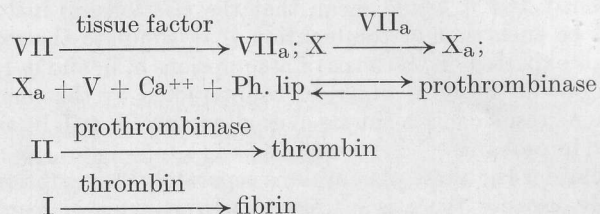
Finally, as long as there is no reasonable objection to assumptions a) and c), and the congruity between theory and practice requires the validity of all three assumptions, we are left with the problem of finding a reasonable basis for assumption b). This requires the discussion of the various reaction schemes that could provide such a basis.

The first reaction scheme of blood coagulation which assigned a well defined place and role to all known coagulation factors was given in the cascade theory of Macfarlane (23) or the waterfall sequence of Davie and Ratnoff (6). The basic feature of this system is the concept of sequential activation. The part of the cascade scheme relevant to the present work (i. e. the extrinsic pathway) reads like this:



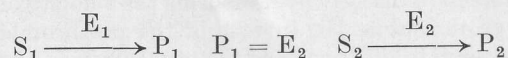
The nature of the prothrombin converting entity (prothrombinase) has been a matter of debate for many years. Its tentative identification with V_a in the above scheme is based on analogy with the other steps. The authors explicitly state that conclusive evidence for the existence is still lacking (6, 23). The possibility of activated factor V being the prothrombin converting principle was recognized as early as 1961 by Straub and Duckert (32); later Breckenridge and Ratnoff provided more evidence for the correctness of this view (4, 5).

In the meantime from the work of Papahadjopoulos and Hanahan (24), Esnouf and Jobin (12) and Hemker, Esnouf, Hemker, Swart and Macfarlane (15) another picture of the prothrombinase emerged. According to these investigators prothrombinase might consist of a complex of factor X_a and factor V adsorbed onto a phospholipid micelle surface, factor X_a being bound via a Ca ion, factor V by a hydrophobic bond. In that case the reaction scheme of the extrinsic pathway would be:



First we will see whether the results of the present communication are compatible with the original cascade scheme.

The simplest unit in an enzyme cascade can be depicted by:



When in both reactions the amount of substrate is so large that the reaction velocity is proportional to the amount of enzyme present, then, as we have shown elsewhere (20), the concentration of P_2 will rise quadratically with time. The simplification achieved by assuming an excess of substrate is probable not justified in practice. Since all more realistic assumptions would introduce more complicated relationships between P_2 and time, and since the argument is developed such that even the simple model used here would react in a more complicated way than is observed in practice, the oversimplification does not invalidate the argument. In such a two step cascade we can make certain deductions about the relation between clotting time and reaction velocity. The moment of clotting (t_c seconds after initiation of the reaction) can be established as the moment at which a critical concentration of fibrin monomere is reached. This critical concentration will be called P_c . For prothrombin estimations, S_1 = factor II, P_1 = thrombin = E_2 , E_1 = prothrombinase, S_2 = fibrinogen, and P_2 = fibrin monomer. We expect the fibrin-monomer concentration to rise quadratically with time with an acceleration proportional to the reaction velocity of the first reaction. If the velocity of the first reaction is v_1 , then:

$$P_2 = \frac{1}{2} v_1 \cdot t^2 \cdot \text{So: } P_c = \frac{1}{2} v_1 \cdot t_c^2 \text{ or } t_c = \sqrt{2 \cdot P_c / v_1}$$

This means that the clotting time has to be linearly proportional to the square root of the inverse of the reaction velocity of the first step. Since we observed a linear relationship between t_c and $1/(\text{factor II})$ i. e. we established that $t_c = a \cdot 1/(\text{factor II}) + b$ it has to follow that

$$2P_c/v_1 = a^2 \cdot 1/(\text{factor II})^2 + 2ab \cdot 1/(\text{factor II}) + b^2.$$

Therefore, the reaction mechanism of factor II conversion would have to be such that the inverse of the reaction velocity is a second degree function of the inverse of the substrate concentration.

Although this remains a possible explanation of the observed data, it must be considered rather improbable because, as will be shown, simpler explanations are possible. Furthermore, reasoning along the same line in a three step cascade mechanism, as would be required to explain the results of the factor V estimations, would require the velocity of factor V conversion to be a third degree function of factor V concentration; factor X conversion velocity would have to be a fourth degree function of factor X concentration etc., which seems to be to remote a possibility to be taken into account seriously.

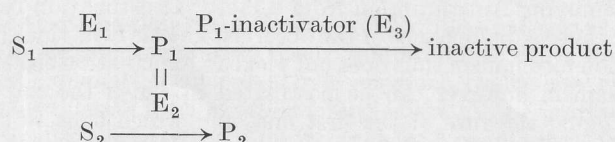
It thus appears that our results are not compatible with the original unmodified cascade scheme. This compels to consider reaction schemes which do account for the experimental facts observed. The basic feature of these schemes has to be the velocity of the final "indicator" reaction (the fibrinogen \rightarrow fibrin conversion) is linearly proportional to the reaction that is rate-limiting in the sequence. Three such schemes will be discussed:

A) The Inactivation Mechanism

If the enzyme ($E_2 = P_1$) produced by the first reaction is inactivated in a subsequent reaction with a velocity that can attain the same magnitude as the velocity of its formation and that is also dependent upon the amount of the enzyme (E_2) present, a steady velocity of E_2 formation will, in the first phase of the reaction, cause a rise of the concentration of E_2 . This rise will lead to the acceleration of the breakdown velocity of E_2 , and this velocity will consequently increase until it equals the velocity of formation. It will be clear that a constant velocity of the first reaction will cause a constant level of the first product and hence a constant velocity of the second reaction. Thus, a steady level of E_2 is the result of a steady velocity of its formation.

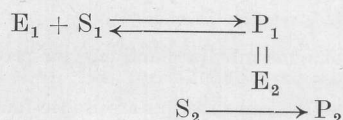
Preliminary mathematical studies indicate that with this mechanism it is not even necessary for P_1 to reach its steady level for the formation velocity of P_2 to become proportional to the formation velocity of P_1 .

This postulate has the advantage to assign a place to the physiological anti-coagulants known to be present in normal plasma (1, 2, 7, 8, 10, 11, 13, 21, 22, 29, 30, 31).



B) The Equilibrium Mechanism

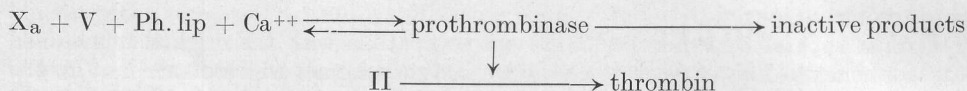
If P_1 is a stoichiometric product of E_1 and S_1 rather than a product derived from S_1 by the action of E_1 , and if the reaction by which P_1 is formed is a reversible one, the condition that P_1 be broken down at a pace dependent upon its concentration is fulfilled as it is in the inactivation mechanism, but in an essentially different reaction scheme:



It seems possible that a combination of the inactivation and the equilibrium mechanism plays in the formation of prothrombinase.

According to the modification of the cascade scheme discussed above, prothrombinase is a stoichiometric product of factor X_a , factor V, phospholipid and Ca^{++} (12, 15, 24). The interaction of these four factors very probably is a reversible one (12, 15, 19). It is common knowledge that the prothrombinase activity in plasma readily disappears. Probably a plasma factor is responsible for this inactivation (8, 11, 31).

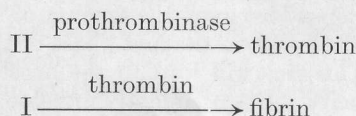
The reactions in which prothrombinase takes part according to these publications can be summarized in the following scheme:



This is essentially a combination of the theoretical schemes A and B discussed above, and thus would be consistent with the experimental facts observed.

It must be concluded that the modification of the cascade scheme is in better agreement with our experimental results than the original scheme is.

This modification may provide an explanation for the kinetic behaviour of the middle part of the reaction sequence but it would leave us with a two step cascade in the lower region viz:



This would still introduce a non rectilinearity of the plot of clotting time versus the inverse of prothrombin concentration in a factor II estimation, as well as non-rectilinearity in a plot of clotting time against the inverse of prothrombinase concentration or any of the other plots discussed. All plots however were observed to be linear.

An equilibrium mechanism obviously fails as an explanation in this case, because the reaction is known to be irreversible. The inactivation mechanism is feasible because thrombin is known to react with Antithrombin III to form an inactive product (21). Removing Antithrombin from a factor II estimation reaction mixture did not however cause a disturbance of the observed kinetic behaviour (Fig. 10).

The inactivation mechanism thus does not yield an explanation either.

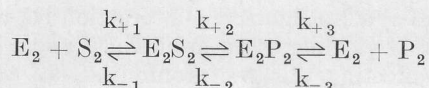
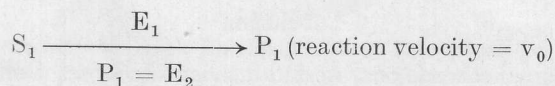
A third mechanism however can be postulated in which the rate of the second reaction would equal the rate of the first one, and which thus would explain our results viz.:

C) The Transient State Mechanism

If S_2 is present in high excess, every molecule of E_2 that enters the reaction medium via the first reaction will immediately be converted into the E_2S_2 complex¹⁾ with the velocity of the formation of E_2 . The E_2S_2 complex is transformed into the E_2P_2 complex. If k_{-1} is small or if the concentration of S_2 is high, v_{-1} will be negligible compared to v_{+1} and the concentration of E_2S_2 will build up until v_{+2} equals v_{+1} ²⁾.

1) With these complexes the enzyme-substrate and enzyme-product complexes as postulated in classical enzyme kinetics are meant (9).

2) To indicate reaction velocities the same subscripts are used as for the indication of the constants.



Now, if there is a negligible amount of free E_2 , v_{-3} will be near zero and v_{+3} will approach v_{+2} ; thus, it will approach v_0 . This reasoning demonstrates that for some period in the transient state of the reaction, i. e. in the interval before the steady-state situation is reached, it will be possible for the rate of formation of P_2 to equal the rate of formation of E_2 . A concise treatment of the enzyme kinetics involved and an experimental approach to their application with regard to the fibrinogen-fibrin conversion are being prepared in our laboratory at the moment. From the above it is clear that this course of events will be favoured by 1. a great excess of S_2 ; 2. a large affinity of E_2 to P_2 ; and 3. conditions tending to render v_{-3} very small. All three these conditions seem to be fulfilled in the thrombin-fibrinogen interaction under our circumstances because: a) fibrinogen appears to be present in large excess (28); b) thrombin has a strong affinity for fibrin (c. f. the Antithrombin I action of fibrin); and c) as P_2 is taken away from the reaction medium as an insoluble polymer; the concentration of P_2 will always remain low, and therefore v_{-3} will tend to remain small. At the moment the transient state mechanism seems to provide a logical explanation for the behaviour of the last part of the sequence, although it has by no means been proven.

In the preceding reaction scheme X_a occurs as an entity, whereas the results mentioned in this article were obtained by variation of the concentration of factor X and factor VII and therefore by variation of the velocity of X_a production. This too can be explained by the fact that the formation rate of prothrombinase rapidly equals the appearance rate of X_a , so that, when factor V and phospholipid are in excess the level of X_a in the reaction medium will rise until the formation rate of prothrombinase equals the formation rate of X_a .

Preliminary results of a mathematical treatment of this situation show that a rectilinear relationship will be observed when the observation time of the over-all reaction (i. e. the clotting time) is of the same order of magnitude as the time needed by the various labile intermediates to reach equilibrium. In other words, the more or less mechanistic explanation offered above, in which events are explained by the existence of a steady-state level of labile intermediate (X_a or prothrombinase), need not be entirely realistic to give the same experimental results with the same reaction scheme.

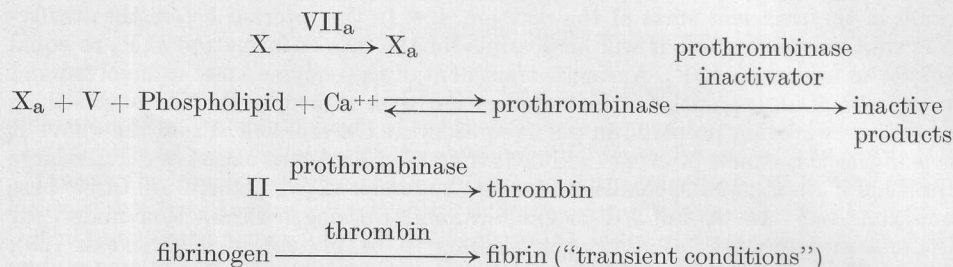
Preliminary experiments in which these systems were simulated with an analog computer have shown that the mechanisms postulated above are indeed feasible.

It might be questioned if it is necessary to introduce the modification of the cascade scheme as we did above, because the presence of an inactivator of each of the activated procoagulants would provide a sufficient basis – at least theoretically – for our findings with the aid of hypothesis A only. This however would mean that inactivators of all procoagulants arising during clotting would have to exist. It is well known that in serum many procoagulants are found in activated form. This possibility thus seems less realistic than the modification of the cascade scheme as given above.

Conclusion

Whereas the original cascade or waterfall mechanism does not offer an explanation of the observed kinetics of the extrinsic system, a somewhat different mechanism does. This mechanism involves a) a stoichiometric-interaction between activated factor X, factor V, and phospholipid yielding a prothrombinase and b) the existence of an inactivation mechanism affecting this prothrombinase. In the interaction between thrombin and fibrinogen, "transient-state" kinetics are thought to be operative, at least until the moment of clotting.

The reaction scheme might thus be:



Summary

Observations of the clotting time in one-stage tests in the extrinsic system show, after suitable correction, that there is a rectilinear relationship between the clotting time and the inverse of the concentration of the rate-limiting factor. This cannot be explained by the "cascade" or "waterfall" mechanism proposed as a scheme for the interaction of the blood-clotting factors concerned. This relationship can, however, be explained by a slightly different mechanism.

Résumé

L'observation du temps de coagulation à l'aide d'un test en un temps dans le système extrinsèque montre, après correction appropriée, qu'il existe une relation linéaire entre le temps de coagulation et l'inverse de la concentration du facteur limitant la vitesse de la réaction. Cela ne peut s'expliquer par la théorie dite en "cascade" ou "chute d'eau" proposée pour interpréter l'interaction des différents facteurs de la coagulation impliqués dans le système extrinsèque. Cette relation peut, par contre, être expliquée par un mécanisme légèrement différent.

Zusammenfassung

Die Bestimmung der Gerinnungszeit mit Hilfe einer Einstufenmethode im »extrinsic«-System zeigt, nach geeigneter Korrektur, eine lineare Reaktion zwischen Gerinnungszeit und reziprokem Wert der Konzentration des die Reaktion limitierenden Faktors. Diese Tatsache kann durch die »Wasserfall«-Theorie nicht erklärt werden, die für die Interpretation der Reaktionen im »extrinsic«-System vorgeschlagen wurde. Diese Interaktion kann jedoch durch einen ähnlichen Mechanismus erklärt werden.

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Addendum

Experiments in which plasma congenitally deficient in factor V was used as a reagent gave essentially the same result as those with artificially factor V deficient plasma described in this article.

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